REPRODUCTIVE MORPHOLOGY, ANATOMY, AND RELATIONSHIPS OF TICODENDRON[1]

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ABSTRACT

Ticodendron is predominantly dioecious. Both male and female inflorescences bear some cymules (or partial inflorescences) on their primary axes. Male partial inflorescences are borne verticillately in clusters of three, each being one- to three-flowered and subtended by a single bract. Staminate flowers have 8–10 or more stamens and lack a perianth. Female partial inflorescences are solitary. Each of them is always one-flowered, although it is subtended by three (one primary and two secondary) bracts, and it produces some scales in each axil of the secondary bracts. In pistillate flowers, concentric bundles are prevalent in the periphery of the pistil wall; the ovary is inferior, bicarpellate, and four-locular with one ovule in each locule; the placentation is axile; the ovules are apical, pendulous, hemitropous, unitegmic, and crassinucellate; the integument is 20–30 cells thick and vascularized by about 20 longitudinally parallel vascular bundles; and the mature seeds have a two-cell-layered endosperm and seed coats composed of thick-walled integumentary cell layers and vascular tissues. In reproductive morphology *Ticodendron* is distinct from any other family but is best placed in Fagales. On the basis of evidence from reproductive morphology and from other sources it is suggested that *Ticodendron* appears to have diverged early from a common ancestor with Betulaceae or Fagaceae.

Ticodendron, comprising only *T. incognita*, which occurs from northern Nicaragua through Costa Rica to central Panama, has long been a mystery plant because of its uncertain identity (Gómez-Laurito & Gómez P., 1989; Hammel & Burger, 1991). The present paper provides a detailed description of floral morphology and anatomy of *Ticodendron* including a few remarks on its embryology, in order to shed light upon the position and affinity of the genus.

MATERIALS AND METHODS

All examined materials were collected at Monteverde, Costa Rica, and fixed with FAA; examined were nearly mature male flowers (*Haber 7072*), young female buds and fruits (*Gómez-Laurito s.n.* in 1986; *Haber 6840, 6868, 7286*). Voucher specimens are deposited at MO and CR.

For anatomical study, single flowers, groups of male and female flowers, and a piece of mature seed were sectioned with a rotary microtome following standard paraffin methods. Before being sectioned, the materials were softened with a mixture of 10 parts of glycerol, 3 parts of 10% Aerosol OT, and 90 parts of water (Schmid & Turner, 1977) as described in Tobe & Raven (1984). Sections cut at 6–10 μm thickness were stained with Heidenhain's hematoxylin, safranin, and fastgreen FCF.

OBSERVATIONS

Ticodendron is mostly dioecious. Male and female inflorescences both appear to be spikes. However, on the basis of anatomical observations discussed later, it has been found that the "spike" is composed of some one- to three-flowered cymules borne on the primary axis. To facilitate discussions in comparing *Ticodendron* with other related taxa, a more comprehensive term, "partial inflorescence" (instead of "cymule"), will be used in the following descriptions (see Abbe, 1974: 164–165, for terminology).

MALE PARTIAL INFLORESCENCES AND FLOWERS

Male partial inflorescences are borne verticillately on the inflorescence axis in clusters of three (Fig. 1). Each male partial inflorescence is subtended by a single primary bract, and is composed of 1–3 staminate flowers basically arranged in a

dichasium. Individual partial inflorescences (as well as individual staminate flowers in the case of three-flowered partial inflorescences) are not readily distinguished from each other, but their distinctness is revealed by vascular bundles. Those supplying each partial inflorescence or each staminate flower depart in separate groups from the vascular cylinder of the inflorescence axis. Staminate flowers have 8–10 or more stamens and lack perianth and pistil, or rarely they have an abortive pistil (see arrow, Fig. 1).

The stamens are tetrasporangiate, and the anthers dehisce longitudinally. Anther walls comprise a fibrous endothecium and a persistent epidermis at maturity. Pollen is two-celled when shed.

FEMALE PARTIAL INFLORESCENCES AND FLOWERS

Female partial inflorescences are solitary, and each is always one-flowered, although it is subtended by one primary and two opposite secondary bracts. These three bracts may fuse basally (Fig. 6B). At each axil of the secondary bracts, some 10 small appendages or scales are produced, each receiving a distinct vascular bundle (Fig. 2). These scales are horizontally scattered in the axil and are attached at somewhat different levels.

Pistillate flowers are composed only of a single inferior ovary with a small, inconspicuous perianth on the top of the ovary (Fig. 3). The flowers rarely have abortive stamens on the inner side of the perianth; the abortive stamens receive a distinct vascular bundle as do ordinary ones. The ovary is bicarpellate with two (rarely three) elongate separate styles and four-locular, each locule containing one ovule (Fig. 4). Styles are stigmatic throughout with elongate and papillate epidermal cells. A narrow adaxial sulcus is present on styles throughout. Judging from the position of the two styles in relation to the inflorescence axis (see Fig. 6C, I), the two carpels are positioned tangentially, not radially.

The floral vascular anatomy of a female flower is presented in Figure 6, which diagrammatically shows the vascular course in a longitudinal section (Fig. 6A) and in selected serial transverse sections (Fig. 6B–I). At and above the level at which the scales are supplied with vascular bundles (Fig. 6B, C), the vascular cylinder, which is composed of many discrete collateral bundles arranged in a ring, irregularly diverges into the peripheral vascular system and the central vascular system (Fig. 6D, E).

The peripheral vascular system is about 15 or 16 aggregates of 4–10 small bundles. Included are a few inverted ones throughout the whole length of flower (Figs. 5, 6D–H). This peripheral vascular system supplies the perianth (and abortive stamens, if present) and the styles (Fig. 6F–I). Each style has three (occasionally four) vascular bundles (Fig. 6I).

The central vascular system is composed of some 20 small collateral bundles, which ascend as placental bundles to supply the ovules (Fig. 6E, F). Ovule placentation is axile.

OVULES

Although pistillate flowers were collected simultaneously with nearly mature staminate ones, the oldest ovules were still in the megaspore dyad stage. Probably more than several weeks pass between pollination and fertilization. The oldest ovules available indicate that the ovule is apical, pendulous, and hemitropous with the micropyle upward, and that it twists somewhat as it grows (Fig. 7). The ovule is crassinucellate. By the megaspore dyad stage, about 4–5 parietal cells lie above the dyad (Fig. 8). Obturators are lacking.

The ovule is unitegmic (Fig. 7), with the integument about 20–30 cells thick and with its tips forming a long narrow micropyle. The integument is vascularized by some 20 longitudinally parallel vascular bundles (Fig. 9), which are derived from a raphe bundle via its post-chalazal branching. The integument is longitudinally ribbed, each rib containing a single vascular bundle (Figs. 9, 10). Outer epidermal cells of the integument are radially elongate and contain rich cytoplasm (Fig. 10).

MATURE SEEDS

Only one of the four ovules in the ovary develops into a mature seed, thus each mature fruit is one-seeded. The embryo of a mature seed is oily and massive with two straight cotyledons. The mature seed has a two-cell-layered endosperm (Fig. 11).

As the seed matures, only the outer tissue of the integument, including a vascular tissue and a surrounding integumentary tissue, persist, while the inner tissue completely degenerates. Thus, the mature seed coat is only about 8–15 cells thick (Fig. 11). Those cells, except for xylem cells of the vascular tissue, are thick-walled and flattened with tanninlike contents. The outer epidermal cells of the integument, which were radially elongate and contained rich cytoplasm in ovular stages, do not show any specialization in the mature seed coat.

DISCUSSION

The above-described inflorescence and floral structures of *Ticodendron* fit within the range of

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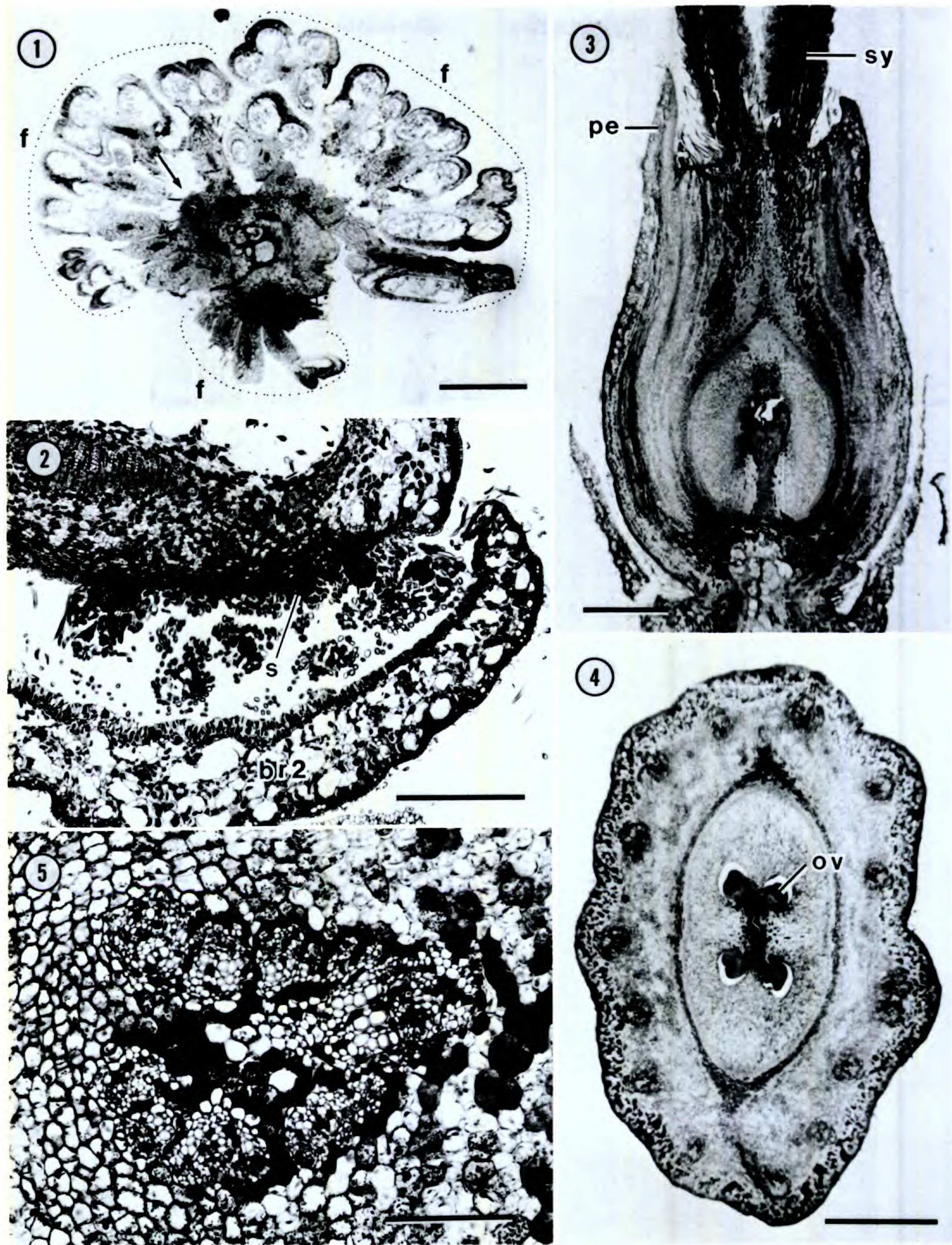
FIGURES 1–5. Anatomy of partial inflorescences and flowers of *Ticodendron*. — 1. Transverse section (TS) of a primary male inflorescence axis showing three male, one-flowered partial inflorescences. Arrow indicates abortive pistil. — 2. TS at the base of female partial inflorescence. Note that some scales are borne at the axil of the secondary bract. — 3. Longitudinal section of female partial inflorescence, which is always one-flowered. — 4. TS of female flower at level of pistil. — 5. TS of concentric bundles prevalent in the periphery of the female flower. Abbreviations: br2, secondary bract; f, single male flower; ov, ovule; pe, perianth; s, scale; sy, style. Scale bars equal 1 mm, 500 μm, 1 mm, 1 mm, and 100 μm, respectively.
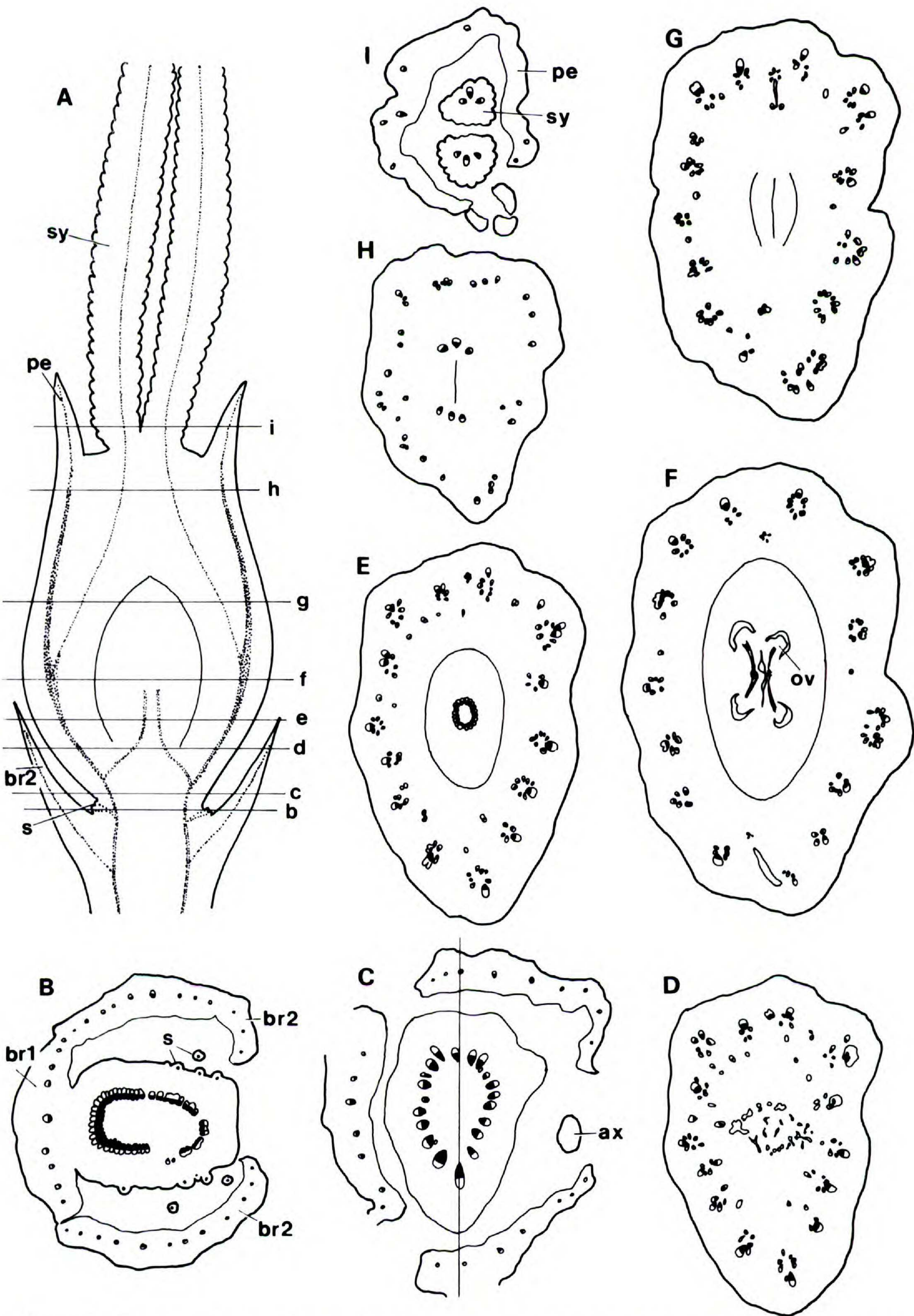
FIGURE 6.   Diagrams illustrating the vascular anatomy of female partial inflorescence and flower in *Ticodendron*. — A. Median longitudinal section through the line presented in C. B–I. Transverse sections at levels marked b–i in A. Abbreviations: ax, suppressed primary inflorescence axis; br1 and br2, primary and secondary bracts; ov, ovule; pe, perianth; s, scale; sy, style.

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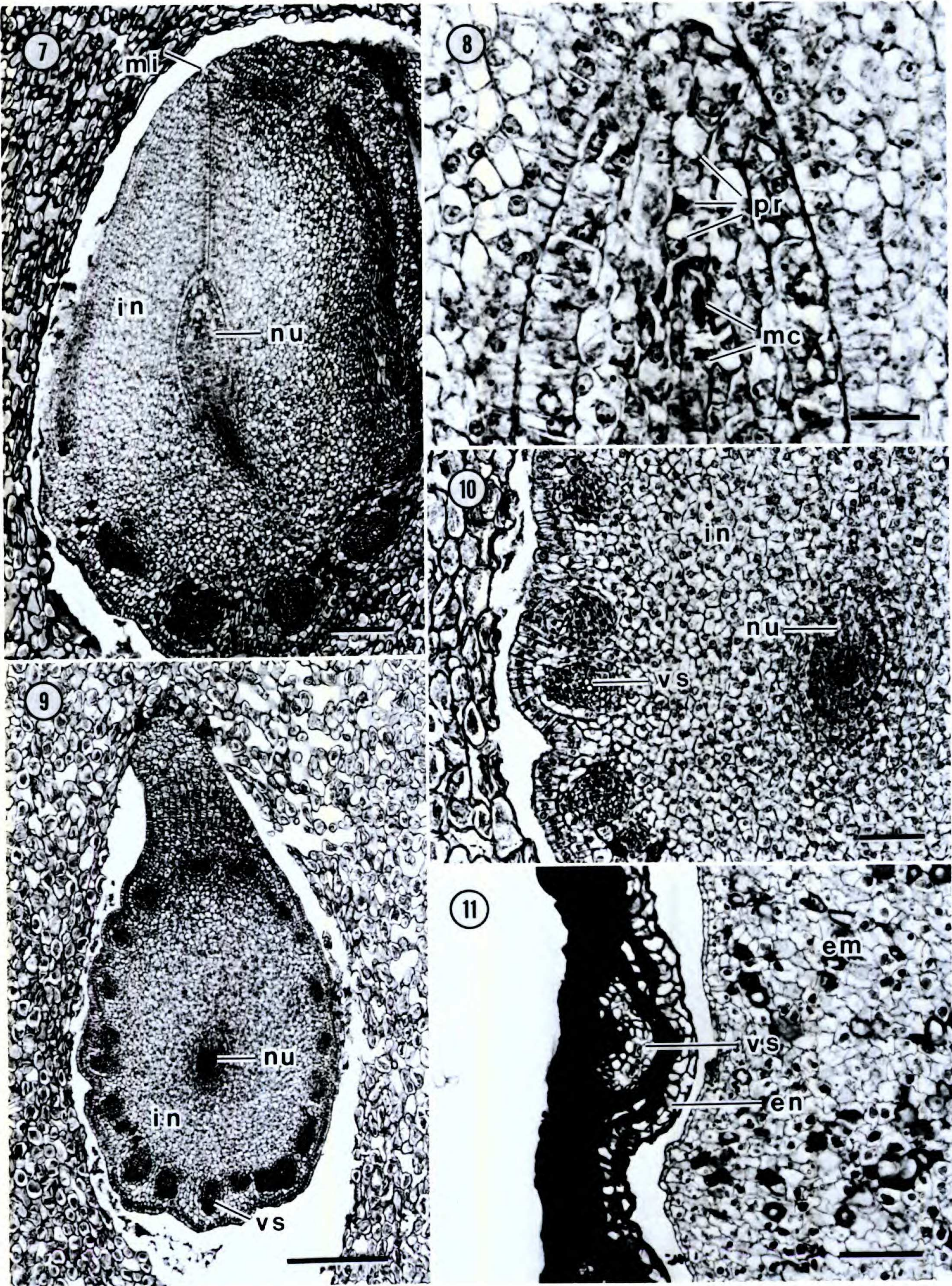
FIGURES 7–11. Anatomy of ovules and seeds in *Ticodendron.*—7. Longitudinal section (LS) of ovule.—8. LS of ovule showing a dyad of megaspores in a nucellus.—9. Transverse section (TS) of ovule showing that the integument is ribbed and contains a vascular bundle in each rib.—10. Enlargement of part of Figure 9, showing a structure of thick integument.—11. TS of mature seed showing seed coat structure. Abbreviations: em, embryo; en, endosperm; in, integument; mc, megaspore; mi, tip of micropyle; nu, nucellus; pr, parietal cell; vs, vascular bundle. Scale bars equal 100 μm, 20 μm, 200 μm, 200 μm, and 100 μm, respectively.

TABLE 1. Comparisons of *Ticodendron* with related families in selected reproductive characters.

Characters[1]	*Ticodendron*	Fagaceae (Fagales)	Betulaceae (Fagales)	Juglandaceae (Juglandales)	Myricaceae (Myricales)
Cupule	Absent	Present	Absent	Absent	Absent
Ovary position	Inferior	Inferior	Inferior	Inferior	Superior
Concentric bundles in pistil wall	Present	?	Present	Present	?
Number of carpels per ovary	2	(2)3(-12)	2(3)	2(-4)	2
Number of locules in ovary	4	(2)3(-12)	2(3)	1	1
Number of ovules in ovary	4	(4)6(-many)	2-4(-6)	1	1
Ovule position	Axile, pendulous	Axile, pendulous	Axile, pendulous	Axile, erect	Basal, erect
Ovule curvature	Hemitropous	Anatropous	Anatropous	Orthotropous	Orthotropous
Number of integuments	1	(1)2	1(2)	1	1
If unitegmic, thickness of integument	20-30 cells thick	4-5 cells thick	4 cells thick	6-10 cells thick	3-7 cells thick
Vascular bundles in integument	Present	Present	Present	Present	Present
Ribbed integument	Present	Absent	?	Absent	?
Mature seed coat	Cells thick-walled	Cells thick-walled	?	Cells thick-walled	?
Thickness of endosperm	2 cells thick	Absent or 1 cell thick	2-4 cells thick	1 cell thick	1 cell thick

[1] References are: Netolitzky (1926), Vaughan (1970), and Corner (1976) for all families (seed coat anatomy); Langdon (1939, 1947) and Poole (1952) for Fagaceae; Wolpert (1910) for Betulaceae; Karsten (1902), Langdon (1939), Meeuse & Houthuesen (1964), Verhoog (1968), and Manning (1978) for Juglandaceae; and Kershaw (1900), Kühn (1927), and Yen (1950) for Myricaceae.

variation for these features shown in Fagales, Juglandales, and Myricales (see Abbe, 1974, for review) and suggest closer affinity of *Ticodendron* with these orders than with other Hamamelididae or members of different subclasses. In Table 1, reproductive features of *Ticodendron*, Fagales, Juglandales, and Myricales are presented.

In general, *Ticodendron* agrees well with Fagales, Juglandales, and Myricales. They share an inferior ovary (except Myricales with a superior ovary), concentric bundles in floral vascular system (though uncertain in Betulaceae and Myricales), bicarpellate ovaries (though frequently tricarpellate in Fagales and Juglandales), unitegmic ovules (commonly bitegmic in Fagales), vascular bundles in the integument (in the outer integument only in Fagales with bitegmic ovules), a similar mature seed coat composed of numerous thick-walled cell layers and vascular tissues, and a thin endosperm in mature seed. *Ticodendron* further coincides with Fagales in having two (or more) ovules per carpel and axile placentation, rather than a uniovulate ovary with basal placentation as in Juglandales and Myricales.

Ticodendron agrees with Myricales in having a longitudinally ribbed integument.

Thus, the reproductive morphology and anatomy of *Ticodendron* indicates a close relationship to Fagales, Juglandales, and Myricales in the Hamamelididae (or "Hamamelidae" sensu Cronquist, 1981). Among Fagales, Juglandales, and Myricales, *Ticodendron* seems to be more at home within Fagales, which have diverse reproductive morphology, rather than in Juglandales or Myricales, both of which are characterized by certain unique apomorphies. Juglandales and Myricales both characteristically have a unilocular bicarpellate ovary with a single orthotropous ovule, all features that are unknown in *Ticodendron* and Fagales. Closer affinities of *Ticodendron* with Fagales are further suggested by evidence from wood anatomy, pollen morphology, and other data, as specified in this volume.

Among Betulaceae, Coryleae may be the closest allies of *Ticodendron*, since Coryleae, like *Ticodendron*, lack a perianth in the male flowers but possess it in the female flowers. Evidence from wood anat-

omy also suggests Coryleae for the closest affinity of *Ticodendron* (Carlquist, 1991). Yet, in *Ticodendron* the ovule is hemitropous with a thicker integument (20–30 cells thick), whereas in Coryleae it is anatropous and probably with a thinner integument (e.g., four cells thick in *Betula*; Wolpert, 1910); *Ticodendron* has scales at the axils of the secondary bracts, which are unknown in Coryleae (and in all other Betulaceae); *Ticodendron* lacks the tertiary bracts that are positioned decussate to the secondary bracts and form by their fusion a characteristic involucre or hull in Coryleae (Abbe, 1935, 1938, 1974); in *Ticodendron* stamens are not deeply divided as in Coryleae (and in all other Betulaceae); and in *Ticodendron* female flowers are solitary, while in Coryleae two lateral flowers of the dichasium usually develop (Abbe, 1935, 1938, 1974). These differences suggest that *Ticodendron* is unlikely to have direct relationships with Coryleae or other Betulaceae.

Nothofagus is the best candidate in comparing Fagaceae with *Ticodendron*. In fact, *Nothofagus* is superficially similar to *Ticodendron* in having exclusively unitegmic ovules, occasionally solitary staminate flowers, partial inflorescences composed of 1–3 pistillate flowers, and occasionally bicarpellate pistillate flowers (for data on *Nothofagus* see Langdon, 1947; Poole, 1952; Abbe, 1974). *Ticodendron*, however, is clearly distinguished from *Nothofagus* (and in general from all other Fagaceae) in having hemitropous ovules with thicker integument (only four or five cells thick in *Nothofagus*; Poole, 1952) and in lacking characteristic cupular valves, lacking a perianth in male flowers, and lacking the tertiary bracts in female partial inflorescences. Those dissimilarities seem to indicate that *Ticodendron* clearly stands out from Fagaceae as well.

Balanopaceae (*Balanops* only), which have been placed by Cronquist (1981, 1988) in Fagales, may also require comparisons with *Ticodendron*. *Balanops* shares with *Ticodendron* a female partial inflorescence comprising a single pistillate flower subtended by a "cupule," an ovary with locules, an anatropous, unitegmic ovule, and drupaceous fruits. However, the "cupule" of *Balanops* is composed of numerous, spirally arranged, deltoid bracts, lacking an elevated axial structure characteristic of Fagaceae (Hjelmqvist, 1948). Its homology with the cupule of Fagaceae is doubted (Cronquist, 1981). Even if the bracts of the "cupule" of *Balanops* are comparable with the scales of *Ticodendron*, the former encloses the whole of the pistillate flower, while the latter is restricted only at the axils of the secondary bracts. *Balanops* is clearly distinct from *Ticodendron* in having a basal, erect ovule and lacking a perianth in the pistillate flower. Hjelmqvist (1948) suggests on the basis of comparison in the "cupule" structure that *Balanops* probably stands nearest Juglandales and Myricales. Carlquist (1980) notes that the wood of *Balanops* is comparable with that of Hamamelidaceae. Zavada & Dilcher (1986) indicate on the basis of similarity cluster analysis using pollen characters that *Balanops* is a sister group of *Nothofagus*. At the present level of our knowledge it is uncertain even whether *Balanops* is related with Fagales in general. *Balanops* is poorly known with respect to many reproductive structures, and an intensive and overall investigation on the genus seems to be desired prior to critical comparisons with putative allies and *Ticodendron*.

In considering evolutionary relationships with Betulaceae and Fagaceae, the presence of the scales in the secondary bract axils of female partial inflorescences of *Ticodendron* may have a key role. Although 1–3-(or 7- or more-)flowered partial male and female inflorescences are prevalent in Betulaceae and Fagaceae (see Abbe, 1935, 1938, 1947), they are generally considered to have evolved from a more complex cymose inflorescence by reduction of branches of higher orders (see Abbe, 1938, 1974). Indeed, reduced outer sterile branches are elaborated as cupular valves in Fagaceae (Fey & Endress, 1983); furthermore, vestigials of presumably reduced outer sterile branches appear as "supernumerary scales" in male partial inflorescences of *Chrysolepis chrysophylla* (Fagaceae) (Hjelmqvist, 1948) or as blinding vascular bundles in female partial inflorescences of *Betula lenta* (Betulaceae) (Abbe, 1935). Although it is uncertain whether the scales of *Ticodendron* are directly relevant to the scales of the cupule of *Nothofagus* or not, their anatomical features are very similar to each other in their cross sectional shape and in receiving a distinct vascular supply (see Langdon, 1947, for cupule-scale anatomy in *Nothofagus*). The scales of the female partial inflorescence of *Ticodendron* may also represent residuals of reduced outer sterile branches of cymose inflorescences.

Regarding nonreproductive characters, Carlquist (1991) showed that *Ticodendron* has primitive wood compared with other Fagales; Feuer (1991) observed that pollen of *Ticodendron* had plesiomorphic (tectate-granular) wall structure shared with Betulaceae, *Nothofagus*, Casuarinales, and Urticales (see Zavada & Dilcher, 1986); and Hickey & Taylor (1991) suggested, on the basis of leaf architectural analysis, that *Ticodendron*

took a more basal position than other Fagales (and Brunelliaceae). *Ticodendron* may be considered an early derivative either from a common ancestor of Fagaceae before the reduced outer sterile branches of the cymose inflorescence were elaborated as the cupular valves, as in modern Fagaceae, or from a common ancestor of Betulaceae before the reduced outer sterile branches were lost completely, as in modern Betulaceae. For more critical discussion of relationships, embryological studies in *Ticodendron, Nothofagus*, and Betulaceae, which are still meager compared with those in other genera or families, are needed.

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